Metastatic Potentiality of Micropapillary and Conventional Histological Patterns: A Comparative Study of 82 Pulmonary Adenocarcinomas

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Background : *The recently described micropapillary pattern (MPP) is potentially a strong unfavorable prognostic marker for adenocarinoma of the lung. None of the previous studies compared the association to nodal metastasis between conventional histological patterns and MPP.*

Method : *Histological patterns (1=solid, 2=poorly formed acinar, 3=well formed acinar, 4=papillary, 5=bronchioloalveolar), and MPP were semiquantitatively evaluated in 82 pulmonary adenocarcinomas and correlated with nodal status.*

Results : *Mean percentages of pattern 1 and 2 are higher in node positive (N+) group (33.9% vs 19.3%, p=0.046; and 20.8% vs 13.4%, p=0.19, respectively). Analysis of the combined amount of pattern 1 and 2 revealed increased statistical significance (54.6% vs 32.5%, p=0.007). Mean percentages of pattern 3, 4 and 5 tended to be lower in N+ group (22.8% vs 29.4%, p=0.24; 2.8% vs 6.2%, p=0.33; and 17.9% vs 31.2%, p=0.053, respectively). Analysis of the combined amount of pattern 3,4 and 5 showed increased statistical significance (43.3% vs 66.8%, p=0.005). Mean percentage of MPP was higher in N+ group (28.3% vs 11.3%, p=0.0007) after excluding the cases with more than 80% percent of pattern 1 and 2. The criterion of MPP ≥ 20% or combined pattern 1 and 2 >50% of tumor is strongly associated with nodal metastasis (p=0.0015). Pattern 1 has the highest rate of correspondence of having a similar pattern in metastases (18/26, 69.2%), followed by MPP (10/19, 52.6%), and pattern 2 (12/23, 52.2%).*

Conclusion : *MPP has comparable metastatic impact to the solid and the poorly formed acinar patterns and it is prognostically informative to document the presence or absence of the solid plus poorly formed glandular pattern > 50% and MPP ≥ 20% when histological subtype is evaluated.*

Keywords : Lung, Adenocarcinoma, Micropapillary, Histological subtypes, Metastasis, Prognostic marker

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Pulmonary adenocarcinoma is currently the most common cancer of the lung. Although, the TNM staging system was shown to be the most accurate prognostic predictor for non-small cell lung cancer, more and more prognostic indicators, and biomarkers are revealed (1-4). This does not only result in more accurate prognostication but it also facilitates further understanding of the oncogenesis and malignant progression of pulmonary adenocarcinoma. There are studies covering various clinical, histological and molecular markers that are correlated with poor survival and metastatic capability of pulmonary adenocarcinoma. Recently, one histological pattern, namely, micropapillary pattern (MPP) is shown to be an unfavorable prognostic marker for early stages of adenocarinoma of the lung (5,6). However, there has not been any study that compared MPP with other pre-existing histological patterns previously defined by WHO (bronchioloalveolar, acinar, papillary and solid with mucin) (7). The objective of this study was to analyze and compare the extent of association each histological pattern has to nodal metastasis, particularly to understand whether MPP possesses more or less metastasis impact relative to the pre-existing conventional histological subtypes.

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Material and Method

The criterion for inclusion of the specimen in the study included all conventional (non-salivary gland type) pulmonary adenocarcinomas that underwent lobectomy, pneumonectomy or wedged resection with available concurrent lymph node dissection in King Chulalongkorn Memorial Hospital. The exclusion criteria were as follows: history of adjuvant chemotherapy or radiation prior to surgery, evidence of extrapulmonary primary (from clinical history, histology or immunohistochemical study), recurrent tumor, massive necrosis, and poorly preserved morphology. Cases read as negative of all lymph node(s) without hilar or peribronchial lymph node included in specimens were also excluded. From 2000 to 2003, the criteria recruited 82 pulmonary adenocarcinomas.

The cases were classified into two groups: lymph node positive (N+) group and lymph node negative (N-) group. The demographic data of the patients, tumor size, and its location were recorded. The tumor that was located less than 3 cm from the hilar bronchial resection margin was either defined as central or close to the hilus in location. All cases were reviewed for histological parameters. The nuclei were subsequently classified into three grades, namely: 1, 2, and 3, according to their size; the irregularity of the nuclear membrane, and the size of the nucleolus. The predominant grade of the nucleus was also recorded. The percentage of each histological pattern presented in each tumor was estimated by examining hematoxylineosin stained paraffin-embedded tissue sections without prior knowledge of the nodal status. The average numbers of blocks examined for tumors that were smaller than or equal to 3 cm and those larger than 3 cm were 3.1 and 4.5 blocks, respectively. The observed histological patterns were adopted after the latest WHO classification of neoplasm of the lung [7] with additional recognition of micropapillary pattern which is defined as glandular structure with multiple small tufts of tumor cells projecting into air space without fibrovascular core. The acinar pattern is further classified into well-formed acinar and poorly formed acinar patterns. The latter is defined as glandular structure with multiple traversing bridges across the gland to a more solid sheet with cribiform lumens. Also recorded were pleural invasion, whether it was present or absent, as well as the histological pattern(s) presenting in metastatic tumor. Statistical analyses were done by Chi-square test or unpaired two-tailed Student's T-test, where appropriate.

Results

The recruited eighty-two pulmonary adenocarcinomas comprised 47(57.3%) males and 35 (42.7%) females. The average age of the patients was 61.5 years (31 to 86 years, n=79). Forty cases (48.8%) had lymph node metastasis. The relationships of the tumor size, location, nuclear grade, and pleural invasion to the nodal status are presented in Table 1. The distance of tumor from the margin of hilar resection that is less than 3 cm, predominance of nuclear grade 3, and pleural invasion were significantly more common in the node positive group (p=0.004, 0.043 and 0.047, respectively). However, tumors that were larger than 3 cm showed no higher number of nodal metastases, with statistical significance, than those that were equal to or smaller than 3 cm (p=0.17).

Table 1. General pathologic characteristics of pulmonary adenocarcinomas and nodal metastases

Characteristics	No. of cases	Node positive	Node negative	p-value
Tumor size				
≤3.0 cm	35	14 (40%)	21 (60%)	NS
> 3.0 cm	47	26 (55%)	21 (45%)	p=0.17
Close to hilus				
No	54	21 (39%)	33 (61%)	
Yes	26	19 (73%)	7 (27%)	p=0.004
Predominant nuclear grade				
Grade < 3	54	22 (41%)	32 (59%)	
Grade 3	28	18 (64%)	10 (36%)	p=0.043
Pleural invasion				
Absent	31	11 (35%)	20 (65%)	
Present	48	28 (58%)	20 (42%)	p=0.047

NS, not significant

Relationships of histological patterns with nodal metastasis (Table 2 and 3)

The results showed that the conventional histological patterns could be broadly divided according to the potentiality of nodal metastasis into two groups. The first group comprised solid and poorly formed acinar patterns, which were present in higher percentages in the N+ tumors than the N- tumors (mean percentage=33.9% (N+) *vs* 19.3% (N-), p=0.046, for solid pattern; and, mean percentage= 20.8% (N+) *vs* 13.4% (N-), p=0.19, for poorly formed acinar pattern). To reduce the influence of the solid pattern that might confound the analysis of the poorly formed acinar pattern, the tumors that had 50% or higher of composed solid pattern were excluded (8 and 14 cases from the N+ group and the N- group, respectively); and statistical significance was noted for the poorly formed acinar pattern (mean percentage= 29.6% (N+) *vs* 15.3% (N-), p=0.048). Furthermore, higher statistical significance was obtained by combining the amount of the solid and the poorly formed acinar patterns together (mean percentage= 54.6% (N+) *vs* 32.5% (N-), p=0.007).

The patterns in the second group comprised BA, well formed acinar, and papillary. Each of them tended to be fewer in nodal positive cases (mean percentage= 17.9% (N+) *vs* 31.2% (N-), p=0.053 for BA; mean percentage= 22.8% (N+) *vs* 29.4% (N-), p=0.24 for well formed acinar; and, mean percentage=2.8% (N+) *vs* 6.2% (N-), p=0.33 for papillary patterns). Statistical significance was obtained by combining the amount of the three patterns together (mean percentage= 43.3% (N+) *vs* 66.8% (N-), p=0.005).

Classifying the tumors after the predominant pattern (Table 3) resulted in 2 (2.4%) cases of pure BA, 18 (22.0%) cases of predominantly BA, 19 (23.2%) cases of well formed acinar, 15(18.3%) cases of poorly formed acinar, 4 (4.9%) cases of papillary, 23 (28.0%) cases of solid and 1 (1.2%) case of clear cell patterns. The minority of tumors, classified as pure BA, predominantly BA, papillary, and well formed acinar patterns, had nodal metastases (0/2, 6/18, 1/4 and 8/19, respectively), whereas the majority of the tumors predominantly composed of poorly formed acinar and solid pattern had nodal metastases (10/15 and14/23, respectively). By classifying the tumors into well differentiated tumors (predominantly composed of BA, well formed acinar, or papillary pattern) and poorly differentiated tumors (predominantly composed of solid or poorly formed acinar pattern), significant difference in rate of nodal metastasis was noted (15/43, 35% *vs* 24/38, 63%, respectively, p = 0.011).

Micropapillary pattern (Table 2 and 3)

Because the structures of MPP could not be formed in the area of solid or poorly formed acinar growth pattern, and because the latter two patterns are highly associated with metastasis; therefore, confounding effects from cases with a large amount of the solid and poorly formed acinar patterns were anticipated in the analysis of MPP. To reduce the confounding effect, 21 cases (7 from node negative and 14 from node positive groups) that had more than 80% percent of solid plus poorly formed acinar patterns were excluded when MPP was analyzed. Table 2 shows that the amount of MPP was significantly higher in tumors with nodal metastasis (mean percentage= 28.3% (N+) *vs* 11.3% (N-), p=0.0007).

Using the quantitative data from Table 2 to select a practical cut off point for Chi-square tests, it is shown in Table 3 that the tumors with MPP of more than or equal to 20% have statistically significant association with nodal metastasis (MPP $\geq$ 20%: 15/26, 58% *vs* MPP < 20%: 11/37, 30%, p=0.03). For conventional histological patterns, the cut off point of 50% for the poorly differentiated pattern gives statistical difference in nodal metastasis (poorly formed acinar plus solid patterns > 50%: 22/34, 65% *vs* poorly formed acinar plus solid patterns $\leq$ 50%: 18/48, 38%, p=0.015). A complimentary effect between MPP and the poorly differentiated pattern is also noted when either MPP $\geq$ 20% or the solid plus the poorly formed acinar >50% is used as inclusion criterion. This criterion gives the highest statistical significance with p value of 0.0015 (34/56, 61% *vs* 6/26, 23%, respectively).

Correspondence of pattern in primary tumor and metastasis (Table 4)

The solid pattern had the highest rate of correspondence of having a similar pattern in nodal metastases (18/26, 69.2%). The remaining patterns with higher to lower rates of correspondent are MPP (10/19, 52.6%), poorly formed acinar (12/23, 52.2%), and well formed acinar patterns (13/30, 43.3%). The number of cases with papillary (n=4), signet ring cell (n=2) and clear cell (n=1) patterns was too small for interpretation.

Table 2. Amount of histological patterns in pulmonary adenocarcinomas and nodal status (Data displayed in mean percentage with 95% confident interval of means in parentheses)

Histological patterns	Node negative (n=42)	Node positive (n=40)	P-value
Solid	19.3% (9.3-29.3%)	33.9% (23.6-44.1%)	0.046
Poorly formed acinar	13.4% (5.7-21.1%)	20.8% (12.9-28.6%)	0.19
Solid + poorly formed acinar	32.5% (21.3-43.7%)	54.6% (43.2-66.1%)	0.007
Micropapillary *	11.3% (5.2-17.4%)	28.3% (21.0-35.6%)	0.0007
Bronchioloalveolar	31.2% (21.8-40.6%)	17.9% (8.2-27.5%)	0.053
Well formed acinar	29.4% (21.6-37.4%)	22.8% (14.7-30.8%)	0.024
Bronchioloalveolar + well formed acinar	60.6% (49.6-71.6%)	40.6% (29.4-51.9%)	0.014
Bronchioloalveolar + well formed acinar + papillary	66.8% (55.6-78.0%)	43.3% (31.9-54.9%)	0.005

* Excluding cases with combined solid and poorly formed acinar patterns more than 80% percent (node negative, n=35; node positive, n= 26)

Table 3. Relationship of histological patterns and nodal metastases of pulmonary adenocarcinomas

Histological patterns	No. of cases	Node positive	Node negative
Classified by the predominant pattern (* and # p=0.011)			
Well differentiated *	43	15 (35%)	28 (65%)
Pure BA	2	0 (0%)	2 (100%)
predominantly BA	18	6 (33%)	12 (67%)
Papillary	4	1 (25%)	3 (75%)
Well formed acinar	19	8 (42%)	11 (58%)
Poorly differentiated #	38	24 (63%)	14 (37%)
Poorly formed acinar	15	10 (67%)	5 (33%)
Solid	23	14 (61%)	9 (39%)
Others: Clear cell	1	1 (100%)	0 (0%)
Classified by amount of pattern(s)			
Solid+poorly formed acinar > 50% (p=0.015)			
yes	34	22 (65%)	12 (35%)
no	48	18 (38%)	30 (62%)
Micropapillary pattern (MPP) ≥ 20% (p=0.03) ϕ			
yes	26	15 (58%)	11 (42%)
no	37	11 (30%)	26 (70%)
Solid+poorly formed acinar >50% or MPP ≥ 20% (p=0.0015)			
yes	56	34 (61%)	22 (39%)
no	26	6 (23%)	20 (77%)

ϕ Excluding cases with combined amount of solid and poorly formed acinar patterns more than 80 % of tumor

Table 4. Correspondence of histological patterns in primary tumors and nodal metastases

Histological patterns	No. of cases	Same pattern present in metastases
Well formed acinar	30	13 (43.3%)
Poorly formed acinar	23	12 (52.2%)
Solid	26	18 (69.2%)
Micropapillary	19	10 (52.6%)
Papillary	4	2 (50.0%)
Signet ring	2	1 (50.0%)
Clear cell	1	0 (0%)

Discussion

Since most pulmonary adenocarcinomas have multiple histological patterns, and particular histological pattern of the tumor, even presented in a small amount, may contribute to its prognosis, classification of each tumor according to its predominant pattern may not be the best approach. In order to understand the extent each histological pattern contributes to its prognosis, the quantity of each histological pattern was measured, compared and correlated to its nodal metastasis. In the node positive group, the correspondence of histological patterns found in primary tumors to those in the metastases was examined to evaluate the metastatic potentiality of each pattern. Although, the present study did not correlate the histological patterns to the actual rate of survival, the correlation is made with nodal status, which is one of the most important biomarkers strongly predictive for patient survival [8,9].

The current version of histological typing issued by WHO represents histological diversities of pulmonary adenocarcinoma. However, it may not be the best scheme for prognostic purpose, since there was no significant or obvious prognostic value by using the histological subtype similar to the classification [10]. In contrast, the classification of pulmonary adenocarcinomas into bronchioloalveolar carcinoma (non-invasive neoplasm), and for invasive tumor, into well differentiated and poorly differentiated groups results in better prognostic significance [9-11], as it is also noted in the present study. Because of the following conditions, firstly lung adenocarcinoma usually comprises a varying amount of multiple histological patterns, and secondly, in practice, some patterns can be confused with the others (such as, bronchiolo-alveolar pattern with papillary structures *vs* papillary pattern, or bronchiolo-alveolar pattern with sclerosis *vs* acinar pattern), it can be difficult to classify each tumor uniformly. From the above reasons, and the finding of complimentary effect between the solid and the poorly formed acinar patterns, pulmonary adenocarcinomas can be prognostically informative, practical and highly reproducible to be classified into three categories, namely: 1) bronchioloalveolar adenocarcinoma when the tumor is exclusively composed of BA pattern (nodal metastatic rate=0%); 2) well differentiated adenocarcinoma when the combined amount of solid and poorly formed acinar patterns is 50% or less (nodal metastatic rate=38%); and 3) poorly differentiated adenocarcinoma when the combined amount of solid and poorly formed acinar patterns exceed 50% (nodal metastatic rate = 65%).

Regarding MPP, the present study shows that it is a histological pattern with strong association with nodal metastasis (the second strongest next to the poorly differentiated pattern) even it is present in a low amount. Therefore, it is recommended that the presence or absence of MPP of more than or equal to 20% should be reported. However, it should be noted that the significant effect of MPP on nodal metastasis in this study is noted after excluding cases where there were 80% or more of the solid plus the poorly formed acinar patterns in the tumor, whereas significant effect of the poorly differentiated patterns (solid and poorly formed acinar patterns) can be generally applied. Nevertheless, MPP can serve as an important prognostic parameter, especially in the cases of smaller tumors where the solid plus poorly formed acinar patterns commonly compose less than 80% of the whole tumor.

The rate of concordant presence of the histological pattern in metastases is the highest for the solid pattern and lower for MPP, the poorly formed acinar, the papillary and the well formed acinar patterns, respectively. These data reflect the higher metastatic potential of the poorly differentiated pattern and MPP compared to the other patterns. The present study also demonstrates the association of tumor location, i.e. whether it is close to or far from the hilus, the predominant nuclear grade 3 and pleural invasion as previously observed [8-12]. In addition, greater amounts of the poorly differentiated pattern are present in the larger tumor (mean solid plus poorly formed gland patterns = 31.7% for tumor $\leq$ 3 cm *vs* 51.9% for tumor > 3 cm, p=0.016), as noted by others [10].

In conclusion, the study quantitatively evaluated conventional histological patterns and the recently described MPP in correlation with nodal metastasis. It shows that MPP has comparable metastatic impact when compared to the solid and the poorly formed acinar patterns, the two histological patterns most strongly related to nodal metastasis. The present study further suggests that, to obtain the highest statistically significant relationship to nodal metastasis, it is practical, useful and reproducible to document the presence or absence of the solid pattern plus the poorly formed glandular pattern of more than 50%, and MPP of more than or equal to 20% when histological subtype of pulmonary adenocarcinoma is evaluated.

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การศึกษาเปรียบเทียบการแพร่กระจายสู่ต่อมน้ำเหลือง ของรูปแบบไมโครปาปิลารี และรูปแบบทางเนื้อเยื่อทั่วไป ในมะเร็งปอด อะดีโนคาร์ซิโนมา 82 ราย

ชวิษฐ์ จันทรานุวัฒน์

ศึกษาเปรียบเทียบความสัมพันธ์ของรูปแบบทางเนื้อเยื่อทั่วไป (1= solid, 2= poorly formed acinar, 3= well formed acinar, 4= papillary, 5= bronchioloalveolar) และ ไมโครปาปิลารี กับการแพร่กระจายสู่ต่อมน้ำเหลืองในมะเร็งปอด อะดีโนคาร์ซิโนมาจำนวน 82 ราย พบว่าค่าเฉลี่ยปริมาณของรูปแบบ 1 และ 2 สูงกว่า ในกลุ่มที่มีการแพร่กระจาย (33.9% ต่อ 19.3%, p=0.046; และ 20.8% ต่อ 13.4%, p=0.19, ตามลำดับ) เมื่อรวมปริมาณของรูปแบบ 1 และ 2 ด้วยกัน พบนัยสำคัญทางสถิติสูงขึ้น (54.6% ต่อ 32.5%, p=0.007) ค่าเฉลี่ยปริมาณของรูปแบบ 3,4 และ 5 มีแนวโน้มต่ำกว่าในกลุ่มที่มีการแพร่กระจาย (22.8% ต่อ 29.4%, p=0.24; 2.8% ต่อ 6.2%, p=0.33; และ 17.9% ต่อ 31.2%, p=0.053, ตามลำดับ) เมื่อรวมปริมาณของรูปแบบ 3,4 และ 5 ด้วยกัน พบนัยสำคัญทางสถิติที่สูง (43.3% ต่อ 66.8%, p=0.005) หลังจากเนื้องอกที่ประกอบด้วยรูปแบบ 1 และ 2 เกิน 80% ถูกคัดออก พบค่าเฉลี่ยปริมาณของไมโครปาปิลารีสูงกว่าชัดเจน ในกลุ่มที่มีการแพร่กระจาย (28.3% ต่อ 11.3%, p=0.0007) พบนัยสำคัญทางสถิติสูงที่สุดเมื่อใช้ ไมโครปาปิลารี ณ 20% หรือ ปริมาณรวมของรูปแบบ 1 และ 2 > 50% เป็นเกณฑ์ (p=0.0015) รูปแบบ 1 มีอัตราการพบรูปแบบเดียวกันในมะเร็งปอด และในต่อมน้ำเหลืองสูงที่สุด (18/26, 69.2%) ตามด้วย ไมโครปาปิลารี (10/19, 52.6%) และ รูปแบบ 2 (12/23, 52.2%) โดยสรุป ไมโครปาปิลารีมีความสัมพันธ์ต่อการแพร่กระจายสู่ต่อมน้ำเหลืองสูงใกล้เคียงกับรูปแบบเนื้อเยื่อที่มีพัฒนาการต่ำ เพื่อประโยชน์ทางการพยากรณ์โรค ควรรายงานการพบปริมาณรวมของรูปแบบ 1 และ 2 > 50% หรือ มีรูปแบบไมโครปาปิลารี ณ 20% ในการแบ่งย่อยชนิดเนื้อเยื่อของมะเร็งปอด อะดีโนคาร์ซิโนมา